In vivo estimation of gluconeogenesis

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Chapter 1

General Introduction

and Outline of the Thesis

Glucose is the most important metabolic fuel that sustains the continuous processes in all living organisms. Thus, maintaining a constant supply of glucose is essential for their normal functions and survival. Under normal conditions the blood glucose concentration is maintained within a very narrow range in mammals both during absorptive and post absorptive states. This is accomplished by maintaining a balance between the enzyme controlled glucose synthesis and hormone regulated glucose uptake through their mutual and complex interactions.

The liver plays a predominant role in glucose homeostasis because of its ability to produce glucose via gluconeogenesis and glycogenolysis during post absorptive conditions and remove glucose from the blood stream during absorptive conditions. Since the amount of glucose produced and utilized in the kidneys are more or less equal except during acidotic and prolonged fasting conditions, they do not or contribute only negligible amounts to the total whole body glucose production (1; 2). Both hepatic and muscle glycogen stores are exhausted within a few hours of food deprivation in infants and several hours in adults. As a result glucose production in the liver via gluconeogenesis is crucial for extra-hepatic tissues during post absorptive conditions.

Gluconeogenesis plays an important role in glucose homeostasis by producing glucose from gluconeogenic substrates either mobilized from endogenous sources via lipolysis and proteolysis or made available from ingested food. Gluconeogenesis also facilitates the utilization of gluconeogenic precursors such as pyruvate and lactate generated during glucose utilization (glycolysis). The gluconeogenic pathway is the functional reversal of the glycolytic pathway regulated by the activation/deactivation of certain key enzymes via complex mechanisms involving glucose regulatory hormones

such as insulin, glucagon, cortisol, growth hormone and epinephrine, and blood glucose concentration.

Lack of ability to control blood glucose concentration within a narrow range is an indication of an imbalance between the rates of glucose production and glucose utilization and is associated with conditions such as diabetes, infection, post surgery etc (3-10). Therefore, methods and techniques that enable us to obtain quantitative estimates of the rates of gluconeogenesis and glycogenolysis are essential to investigating glucose metabolism during various physiological and pathological conditions. The quantitative *in vivo* estimates of total gluconeogenesis were difficult because of the complexity of the gluconeogenic pathway until various tracer methods were developed.

In vivo estimation of Gluconeogenesis

Various approaches such as estimation based on arterial-venous differences, radioactive or stable isotope labeling of individual substrates have been employed to determine the proportion of glucose release attributable to gluconeogenesis. About 15 years ago three approaches using compounds labeled with stable isotopes with the potential to accurately estimate total gluconeogenesis were published (11-15). However, each of them has both strengths and limitations. These methods are either based on mass isotopomer distribution analysis during the infusion of uniformly ^{13}C labeled stable isotope tracers of glucose/ glycerol or on the deuterium labeling in glucose carbon after the ingestion of deuterium oxide. The approach developed by Landau et al.(15) is widely considered as the golden standard to estimate gluconeogenesis.

Among the various approaches to measure gluconeogenesis using compounds labeled with stable isotopes, methods using deuterated water are simple and straightforward (11-15). The principle of this model is based on the hydrogen/deuterium labeling of new glucose from body water during gluconeogenesis (15). Body water is the precursor pool for the hydrogen/deuterium on the glucose carbon skeleton. This method first reported by Landau *et al.* is based on the deuterium labeling at glucose carbon 5 accounting for all the substrates entering the gluconeogenic pathway and thus, gives an estimate of total gluconeogenesis (15). However, the analytical difficulty and large plasma volume requirement have resulted in a limited use of this method. Therefore, a simple alternative method requiring less sample and expertise is necessary and can potentially promote research in the field of glucose metabolism.

There are many observations suggesting comparable deuterium labeling on various glucose carbons during the gluconeogenic pathway. The ^{2}H-NMR spectra of glucose synthesized from fructose-6-phosphate and dihydroxyacetone phosphate in $^{2}H_2O$ appears to have comparable deuterium labeling in glucose C-1,3,4,5,6 and C-3,4,5 respectively (16). The amount of deuterium labeling at glucose C-3 and 5 are reportedly comparable because of the similarity in deuterium labeling at the trios phosphate level of the gluconeogenic pathway (17). It has been reported that the incorporation of ^{3}H on C-1 of glucose formed by hepatocytes incubated with $^{3}H_2O$ using different substrates was essentially half that on C-6 (two hydrogen on C-6) suggesting that the primary mechanism of labeling is similar between C-1 and C-6 (18). The extensive tritium incorporation in C-3, 4 and 5 of glucose when incubated with a variety of gluconeogenic substrates indicates that the labeling occurs throughout the gluconeogenic process (18).

Several investigators have reported measurements of gluconeogenesis based on ^{2}H-enrichment at C-3, C-5 or C-6 (14; 15; 19-21). Deuterium labeling at the same or different carbons by a single or multiple substrates (15; 19; 21-23), repeated cycling of substrates and a series of isomerization/equilibration reactions during the gluconeogenic pathway should result in a nearly equal distribution of deuterium labeling on glucose C-1,3,4,5 and 6. Deuterium labeling at glucose C-2 is complete due to the extensive glucose-6-phosphate to fructose-6-phosphate isomerization process and is not a reflection of gluconeogenic process (14; 18). Therefore, measurement based on the average deuterium enrichment at glucose C-1, 3, 4, 5 and 6 should provide an accurate estimate of gluconeogenesis.

In our preliminary experiments, we studied two children with glycogen storage disease Type-I using $^{2}H_2O$ to demonstrate that the average enrichment method does not include any labeling due to non-gluconeogenic exchange reactions. The glycogen storage disease Type-I patients lack the glucose-6-phosphatase enzyme, and therefore, are unable to release free glucose into the plasma pool via gluconeogenesis. We observed that fractional gluconeogenesis was essentially zero. This demonstrated that non-gluconeogenic exchange reactions or isotope effects do not result in detectable amounts of deuterium labeling on glucose at the level of deuterium enrichment in body water used in our studies.

Thus, a new easy and straightforward method based on the average deuterium enrichment on glucose C-1,3,4,5 and 6 could potentially provide a reasonable estimate of total gluconeogenesis (including the contribution from all substrates) and that would be useful to many investigators.

Measurement of gluconeogenesis in preterm infants

Disturbed glucose homeostasis is associated with increased morbidity and mortality in very premature infants during the early weeks of life (24-28). Because of low tolerance for enteral feeding, preterm infants are dependent on total parenteral nutrition (TPN). However, these infants also have a reduced tolerance for parenteral glucose, which often results in hyperglycemia. Detailed knowledge about the physiology of the glucose metabolism in these infants is necessary to reduce the risk of hypo/hyperglycemia and to optimize their energy intake for normal growth during this crucial period of life.

According to recent clinical routines, very premature infants often receive TPN providing glucose at rates exceeding normal infant glucose turnover rate. However, there are no data on the effect of this nutritional regimen on total gluconeogenesis and glycogenolysis in very premature infants. Thus, it is not known if gluconeogenesis is affected by the increased amount of glucose given as a part of TPN or if it contributes to the disturbed glucose homeostasis. Furthermore, information on potential hormonal factors regulating gluconeogenesis under these conditions is crucial to optimize nutritional strategies in this population. These issues are addressed in this thesis.

Gluconeogenesis during Lactation in humans

Lactating women have increased glucose demands during fasting to meet the substrate needs for lactose synthesis. Previously it was shown that during a 24 h fast, this higher demand was met by increased glycogenolysis (29). However, it is not known how lactating women adapt to the increased glucose demands during extended fasting periods.

As a part of this thesis, we sought to determine whether lactating women conserve plasma glucose by increasing gluconeogenesis during extended periods of fasting.

Effect of Ghrelin on glucose regulation in mice

Obesity is a global epidemic and is associated with significant risk for metabolic diseases (30-32). Metabolic abnormalities such as type 2 diabetes are of great concern and their economic burden on society is alarming. Diabetes is associated with disturbed glucose homeostasis resulting from imprecise control of glucose production and utilization (3-5). More research is needed to find effective alternatives to treat diabetes and to control this worldwide health crisis.

In addition to weight loss, remission of type 2 diabetes has been recently reported after bariatric surgery procedures. Both short term and long term effectiveness of these procedures in treating diabetes in the obese population has received immense interest (33-35). Although lowering of plasma ghrelin concentrations after bariatric surgery procedures has been suggested as the potential mechanism for this altered glucose metabolism [21], the association between improvement of glucose metabolism and lower ghrelin concentration remains to be determined. Glucose kinetic studies in the absence of ghrelin or its receptor using transgenic mice models (36-39) provide an opportunity to investigate the effects of ghrelin on glucose metabolism and have been used in this thesis.

Application of subcutaneous infusion technique to perform *in vivo* human metabolic studies

Metabolic studies utilizing stable isotope tracers in humans have typically utilized intravenous tracer infusions and venous blood sampling and therefore, are always carried out in an inpatient or outpatient clinical research setting. However, such study conditions dramatically decrease the subject's normal activity during the period of study and thus, do not represent real-life conditions. The use of a subcutaneous tracer infusion and a "finger stick" blood sampling method is a possible alternative to study glucose kinetics under more real-life conditions. We have used a simultaneous intravenous and subcutaneous infusion of two glucose tracers and corresponding blood sampling which provided an opportunity to evaluate the application of this method in future metabolic studies in real life situations.

Collectively, the objective of this thesis is to develop a simple, yet accurate and reproducible method to measure gluconeogenesis, and estimate *in vivo* rates of gluconeogenesis applying the new and other methods in different populations under various conditions to address different issues in the field of glucose metabolism.

Outline of the thesis

The first aim of this thesis is to develop a method to estimate rates of gluconeogenesis that is straightforward, cost-effective and simple and that requires minimal sample volumes (i.e. applicable to all subject populations) using deuterated water. The following questions are addressed:

- What is the suitable derivative and gas chromatography-mass spectrometry (GC-MS) fragment of glucose that carries the exchangeable hydrogen that best represent the gluconeogenic process?
- How does the new method compare with the hexamethylenetetramine (HMT) method reported by Landau et al. (15) when measured under different physiological conditions in different populations? (Chapter 2)
- What is the reproducibility of data on gluconeogenesis obtained by the new method? (Chapter 2)

The second aim of this thesis is to measure gluconeogenesis in very premature infants receiving glucose as a part of routine total parenteral nutrition (TPN) i.e. at rates exceeding their normal glucose turnover rate. Further, this thesis seeks to determine potential factors regulating gluconeogenesis in Extremely Low Birth Weight infants receiving routine total parenteral nutrition. The following questions are specifically addressed:

- Is gluconeogenesis sustained in very preterm infants receiving routine total parenteral nutrition providing glucose at rates exceeding normal infant glucose turnover rate? (Chapter 3)
- What is the contribution of total gluconeogenesis to glucose production in very preterm infants receiving total parenteral nutrition providing glucose exceeding normal infant glucose turnover rate? (Chapter 3)
- Is gluconeogenesis affected by glucose infusion rate, blood glucose concentrations, birth weight or gestational age? (Chapter 3 and 4)

- Is gluconeogenesis affected by a substantial reduction in glucose supply and subsequent changes in insulin or any other potential insulin counter regulatory hormone concentrations? (Chapter 4)

The higher glucose demand in lactating women during a 24 h fast was met by increased glycogenolysis while gluconeogenesis continued at similar rates to that in controls (29). The third aim of this thesis is to determine whether or how lactating women meet the demand of lactose synthesis during an extended period of fasting (without becoming hypoglycemic). The following questions are addressed:

- How do women adapt to their own higher glucose requirement during lactation? And does gluconeogenesis increase to meet the substrate needs for lactose synthesis during an extended period of fasting in lactating women as compared to controls? (Chapter 5)

The fourth aim of this thesis is to determine the effect of the absence of the hormone ghrelin or its purported receptor on insulin sensitivity and glucose production from gluconeogenesis and glycogenolysis in mice. The following questions are addressed:

- What is the effect of the absence of ghrelin or its purported receptor on hepatic and peripheral insulin sensitivity in mice? (Chapter 6)
- Does gluconeogenesis and glycogenolysis change in the absence of ghrelin or its purported receptor during post absorptive conditions in mice? (Chapter 6)

The fifth aim of the thesis is to develop a metabolic study design using subcutaneous tracer infusion in humans that minimally interfere with the normal activity of the subject, such that glucose appearance rate can be measured in real life situations. The following question is addressed:

- Does the data from subcutaneous infusion followed by" finger stick" blood sampling compare well with the established intravenous tracer infusion and venous blood sampling technique? (Chapter 7)

The final section of this thesis (chapter 8) provides a summary, discussion of the main findings of the thesis and future directions of research in continuum to the works accomplished as a part of this thesis.

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